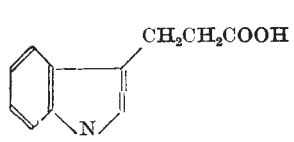
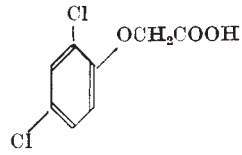


(I)



(II)

With appropriate ring systems, therefore, it would seem that plant growth-regulating activity can be associated with the —CH_2CH_2COOH side-chain provided this possesses adequate stability against β-oxidation within the plant.

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MECHANISMS OF FORMATION OF STEREOISOMERS OF 2,3-BUTANEDIOL DURING MICROBIAL FERMENTATION OF SUGARS

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ALL three isomers of 2,3-butanediol [(L+), D(−) and *meso*] are formed as end-products of the fermentation of sugars by various micro-organisms[1]. The mechanisms concerned with the production of these isomers have not as yet been completely elucidated. The present work is a study of the reactions in several bacteria leading to the formation of different stereoisomers of 2,3-butanediol.

The specific rotations of 2,3-butanediols, isolated from culture media containing glucose after the fermentative growth of the organisms used in this study, are shown in Table 1. During these fermentations pyruvate, formed from glucose, is largely converted to lævorotatory acetoin. Acetoin serves as a hydrogen acceptor, being reduced to 2,3-butanediol as outlined in Fig. 1. Acetone-dried cell preparations or cell-free extracts were used to convert pyruvate to acetoin and the optical rotation of the latter compound was determined in each case (Table 1). Since all samples of acetoin were lævorotatory, the mode of reduction of acetoin to 2,3-butanediol cannot be the same for all the micro-organisms and still yield different isomers of 2,3-butanediol.

The steric specificities of the 2,3-butanediol dehydrogenases were determined by oxidizing *meso*-2,3-butanediol in the presence of oxygen, methylene blue, diphosphopyridine nucleotide and hydroxylamine according to Aubert and Gavard[3]. For each organism the acetoin produced was liberated from its oxime with nitrous acid[4] and the optical rotation determined (Table 1). Cleavage of the acetoin oxime has been shown to result in some racemization[4]. It can be seen that the dehydrogenase from *Bacillus polymyxa* oxidized the hydroxyl group in the (−) configuration [(−) dehydrogenase] yielding dextrorotatory acetoin, while the corresponding enzyme from *Aerobacter aerogenes* attacked the hydroxyl group in the (+) configuration [(+) dehydrogenase] yielding lævorotatory acetoin as illustrated in Fig. 2. It is thus possible to explain the formation of the D(−) isomer of 2,3-butanediol from glucose by *B. polymyxa* since the carbonyl group of lævorotatory acetoin, the immediate precursor, is reduced by the (−) dehydrogenase to a hydroxyl group in the (−) configuration (Fig. 1). Similarly, *A. aerogenes* forms 2,3-butanediol which is predominantly the *meso*-isomer since the carbonyl group of lævorotatory acetoin, formed from glucose, is reduced by the (+) dehydrogenase to a hydroxyl group in the (+) configuration (Fig. 1).

Table 1

Organism	Specific rotation of 2,3-butanediol formed from glucose (deg.)	Specific rotation of acetoin formed from pyruvate (deg.)	Specific rotation of acetoin formed by oxidation of *meso*-2,3-butanediol (deg.)
B. polymyxa	− 10·6*	− 96	+ 73·5
A. aerogenes	+ 0·8 to + 1·8†	− 94	− 73·0
Ps. hydrophila	+ 0·94	− 34	− 70·5
B. subtilis	− 5·7	− 78	− 23·5

* The specific rotation of 2,3-butanediol is a function of the amount of water in the sample measured[2]. Values as high as − 13·3° have been reported for the anhydrous D(−) isomer obtained from *B. polymyxa* fermentations which was shown to be at least 98 per cent pure (ref. 1).

† Values taken from ref. 1.

Pseudomonas hydrophila oxidized *meso*-2,3-butanediol to lævorotatory acetoin, as did *A. aerogenes*, and therefore contains a (+) dehydrogenase (Table 1). Acetoin formed from pyruvate by *Ps. hydrophila* had a lower specific rotation (− 34°) than did acetoin formed by *A. aerogenes* (− 94°). The lowered rotation of acetoin from *Ps. hydrophila* has been shown to be due to the presence in these cells of an acetoin racemase, since incubation of optically active acetoin with this preparation resulted in a considerable decrease in specific rotation of the acetoin recovered at the end of the experiment. The high specific rotation of acetoin obtained by the oxidation of *meso*-2,3-butanediol (Table 1) with *Ps. hydrophila* may be accounted for by the fact that acetoin was trapped as the oxime in this experiment and was probably not available as a substrate for the racemase. The presence of acetoin racemase in *Ps. hydrophila* accounts for the production of a small amount of L(+)-2,3-butanediol during the fermentation of glucose. Lævorotatory acetoin, the isomer of this

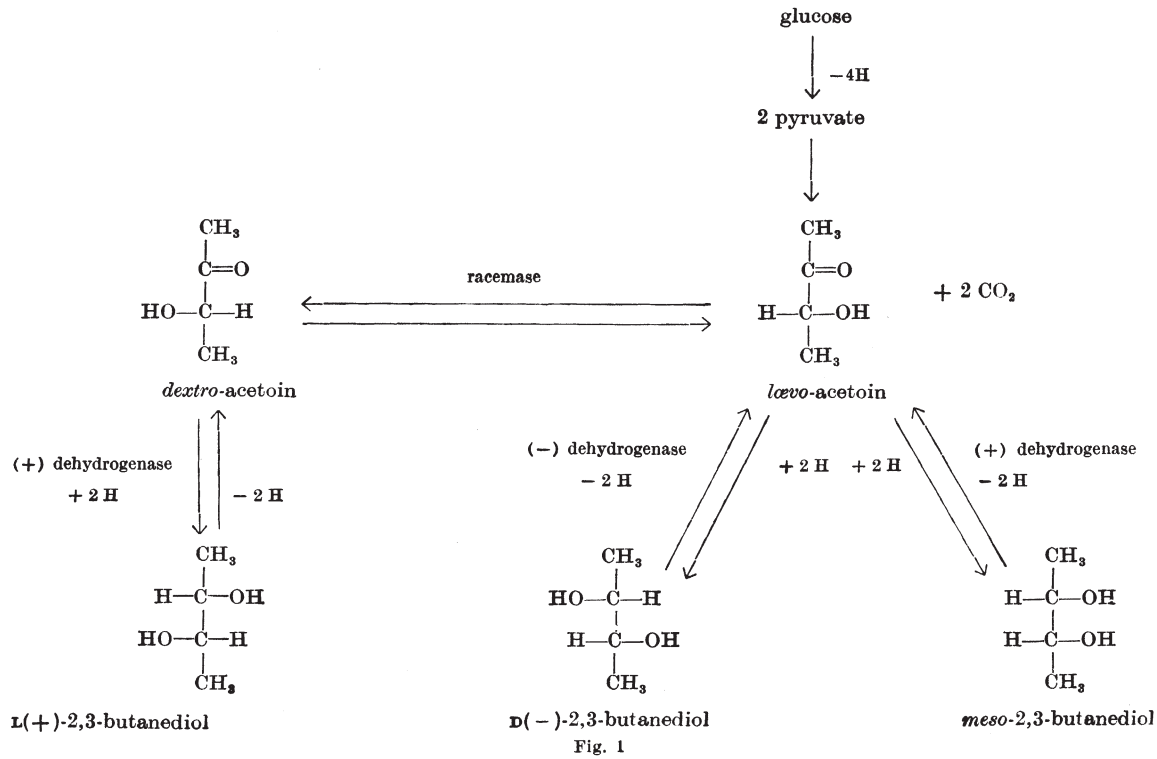

Fig. 1

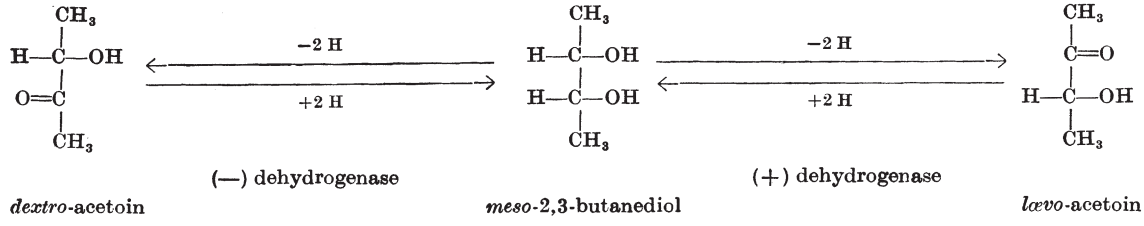

Fig. 2

compound produced from glucose by *Ps. hydrophila*, is reduced to *meso*-2,3-butanediol in the same manner as described above for *A. aerogenes*. The small quantity of dextrorotatory acetoin formed by the action of acetoin racemase is reduced by the same (+) dehydrogenase to L(+)-2,3-butanediol as shown in Fig. 1. The reported formation of some L(+)-2,3-butanediol by various strains of *A. aerogenes* may be due to the presence of acetoin racemase in these species. We have been unable to demonstrate such an enzyme in the *A. aerogenes* preparation studied in this laboratory.

Acetoin produced from pyruvate by *Bacillus subtilis* had a somewhat lower specific rotation than that from *B. polymyxa* and *A. aerogenes* (Table 1). It is possible that there may have been a weak acetoin racemase in the *B. subtilis* preparation. The fact that oxidation of *meso*-2,3-butanediol by *B. subtilis* resulted in the formation of acetoin having a low specific rotation (− 23·5°) was explained by demonstrating that this organism contained a mixture of the (+) dehydrogenase and the (−) dehydrogenase. A crude enzyme preparation from *B. subtilis* could oxidize both D(−)-2,3-butanediol and *meso*-2,3-butanediol. When this preparation was aged at 4° C. for six days it was still active in oxidizing *meso*-2,3-butanediol [(+) dehydrogenase] but showed no activity in oxidizing D(−)-2,3-butanediol [(−) dehydrogenase]. Both enzymes were active after being kept in the frozen state for the same length of time. This finding makes it possible to explain the specific rotation of 2,3-butanediol obtained after fermentation of glucose by *B. subtilis* (Table 1). Lævorotatory acetoin, formed from glucose, is in part reduced by the (−) dehydrogenase to D(−)-2,3-butanediol and in part reduced by the (+) dehydrogenase to *meso*-2,3-butanediol (Fig. 1). A more detailed report of this work will appear elsewhere.

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